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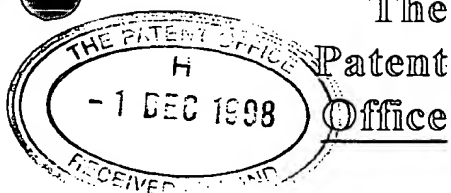
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2.	Patent application number *(The Patent office will fill in this part)*	**01 DEC 1998** **9826359.3**

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3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	GLAXO GROUP LIMITED GLAXO WELLCOME HOUSE BERKELEY AVENUE GREENFORD MIDDLESEX UB6 ONN GB
	Patents ADP number *(if you know it)*	4735870003
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4	Title of the invention	NOVEL RECEPTORS

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Novel Receptors

Field of the Invention

5   The present invention relates to human vanilloid-receptor (hVR) proteins and to related nucleotide sequences, expression vectors, cell lines, antibodies, screening methods, compounds, methods of production and methods of treatment, as well as other related aspects.

10

Background of the Invention

Capsaicin, the irritant in hot peppers and a member of the vanilloid family activates a sub-group of sensory neurons: the nociceptors.
15   These neurons transmit nociceptive and thermoceptive pain information back to pain-processing centres in the central nervous system such as the spinal cord and the brain. They are also sites for the release of pro-inflammatory mediators in the periphery (1). Nociceptors show heterogeneity in their sensitivity to capsaicin.
20   Excitation and prolonged exposure of these neurons to capsaicin is followed by a refractory state known as desensitisation (2) when they become insensitive to capsaicin and other noxious stimuli (3). The long-term response to insensitivity could be explained by death of the nociceptors or destruction of its peripheral terminals (4).
25   Because of the desensitisation phenomenon, capsaicin has been used therapeutically for decades as an analgesic agent for the treatment of pain in a range of disorders (5).

It has been speculated that the endogenous target for capsaicin
30   plays an important function in the detection of painful stimuli. It has been shown by electrophysiological and biochemical studies that capsaicin induces a flux of cations in dorsal root ganglion (DRG) neurons (6,7). Because other vanilloid derivatives show responses in a dose dependent manner (8,9) a receptor is the most likely
35   candidate to explain the mechanism. Therefore, based on indirect

human vanilloid receptor sub-types could provide targets for the development of novel analgesic agents (agonists and antagonists) and agents which may interact with other disorders.

5 Accordingly, it is an object of the present invention to locate and characterise human vanilloid receptors. Other objects of the present invention will become apparent from the following detailed description thereof.

10 **Summary of the Invention**

According to one embodiment of the present invention there is provided an isolated human vanilloid receptor (hVR) protein or a variant thereof. Preferably the hVR protein is an hVR1, hVR2 or 15 hVR3 protein or a variant thereof. In a particularly preferred aspect of the invention the hVR protein has an amino acid sequence as shown in Figure 2.

According to another aspect of the invention there is provided a 20 nucleotide sequence encoding a human vanilloid receptor (hVR) protein or a variant thereof, or a nucleotide sequence which is complementary thereto. Preferably the nucleotide sequence encodes an hVR1, hVR2 or hVR3 protein or variant thereof or a nucleotide sequence which is complementary thereto. Particularly 25 preferably the nucleotide sequence is as shown in Figure 1.

According to another aspect of the invention there is provided an expression vector comprising a nucleic acid sequence as referred to above which is capable of expressing an hVR protein.

30
According to another aspect of the invention there is provided a stable cell line comprising an expression vector as referred to above. Preferably the call line is a modified HEK293, C40 or HeLa cell line.

thereof, under conditions suitable for obtaining expression of the hVR-protein or variant.

Brief Description of the Figures

5

Figure 1 shows the complete nucleotide sequence of the human VR1 including the 2517 open reading frame (base 51- 2568)

Figure 2 shows the nucleotide and encoded amino acid sequence of
10 the human VR1 sequence.

Figure 3 shows the amino acid sequence of the human VR1 gene; the shading denotes predicted trans-membrane-regions (boxed) and the ankyrin binding domains (unboxed). The predicted
15 phosphorylation sites are underlined.

Figure 4 shows multiple alignment of the rat and human VR1 full-length amino acids sequences. The genes are described as: hVR1 for human VR1 and rVR1 for rat VR1.
20

Figure 5 shows a slot blot hybridisation of various tissues and clones with a probe derived from the amino acid coding region of the human VR1 gene.

25 Figure 6 shows a multiple alignment of the amino acids sequences of the various human vanilloid receptors genes identified with the rat VR1 sequence. They are described as: hVR1 for human VR1, rVR1 for rat VR1, 3primehVR2 for the 3' end of human VR2 5primehVR2 for the 5' end of human VR2 and 3endhVR3 for the 3' end of human
30 VR3.

Figure 7 shows the nucleotide sequence of the 5' end of hVR2.

Figure 8 shows the nucleotide sequence of the 3' end of hVR2.
35

Routine methods, as further explained in the subsequent experimental section, can be employed to purify and/or synthesise the receptor proteins according to the invention. Such methods are well understood by persons skilled in the art, and include techniques such as those disclosed in Sambrook, J. et al (28), the disclosure of which is included herein in its entirety by way of reference.

By the term "variant" what is meant throughout the specification and claims is that other peptides or proteins which retain the same essential character of the vanilloid receptor proteins for which sequence information is provided, are also intended to be included within the scope of the invention. For example, other peptides or proteins with greater than about 80%, preferably at least 90% and particularly preferably at least 95% homology with the sequences provided are considered as variants of the receptor proteins. Such variants may include the deletion, modification or addition of single amino acids or groups of amino acids within the protein sequence, as long as the peptide maintains the biological functionality of a vanilloid receptor. This biological functionality can of course be assessed by conducting binding studies with the know vanilloid modulators: capsaicin, capsazepine (12) and resiniferatoxin (11). The term "variant" does, however, exclude the rat VR1 protein which has been previously identified (15).

Human VR1 is preferentially expressed in human dorsal root ganglia (DRG) and relative to hVR2 and hVR3 has the highest sequence homology with the rat VR1. Therefore, hVR1 is likely to be the human orthologue to rat VR1. hVR2 and hVR3 are less similar to rat VR1 and are expressed in a wider range of tissues. Nucleotide sequence analysis of hVR1 reveals a 2517bp open reading frame which encodes an 839 amino acid protein (see Figures 1, 2 and 3). This deduced protein sequence is 90 % identical to the rat VR1 (15) and shares many of its characteristics such as 6 transmembrane regions with an hydrophobic stretch between transmembrane 5 and 6 and an N-terminus which contains 3 ankyrin repeat domains.

which have been modified by insertion of vectors encoding for the receptor proteins according to the invention include the mammalian HEK293T, CHO, HeLa and COS cells. Preferably the cell line selected will be one which is not only stable, but also allows for mature glycosylation and cell surface expression of the inventive receptors.

It is also possible for the receptors of the invention to be transiently expressed in a cell line or on a membrane, such as for example in a baculovirus expression system. Such systems, which are adapted to express the receptors according to the invention, are also included within the scope of the present invention.

In particular, the functional hVR protein may include hVR receptor proteins selected from hVR1, hVR2 and hVR3 and thereof or even other hVR protein subtypes or splice variants which have not yet been identified.

According to another aspect, the present invention also relates to antibodies (either polyclonal or preferably monoclonal antibodies) which have been raised by standard techniques and are specific for the receptor proteins or variants thereof according to the invention. Such antibodies could for example, be useful in purification, isolation or screening involving immuno precipitation techniques and may be used as tools to further ellucidate hVR protein function, or indeed as therapeutic agents in their own right. Antibodies may also be raised against specific epitopes of the receptors according to the invention.

An important aspect of the present invention is the use of receptor proteins according to the invention in screening methods designed to identify compounds which act as receptor ligands and which may be useful to modulate receptor activity. In general terms, such screening methods will involve contacting the receptor protein concerned, preferably hVR1, hVR2 or hVR3, with a test compound and then detecting modulation in the receptor activity, or indeed

Pharmaceutical Sciences, Mack Publishing Company, Eastern Pennsylvania, 17th Ed, 1985, the disclosure of which is included herein in its entirety by way of reference.

5 The compounds may be administered via enteral or parenteral routes such as via oral, buccal, anal, pulmonary, intravenous, intraarterial, intramuscular, intraperitoneal, topical or other appropriate administration routes.

10 The present invention will be further explained, by way of example, in the appended experimental section.

Experimental

15

Identification of related human ESTs (Expressed Sequence Tags) (19) to the rat VR1 sequence by in silico analysis:

The full-length rat VR1 amino acid sequence (15) was used as a
20 query sequence using the tBlastn (20) alignment program to identify related human genes in the dbEST (21) and Incyte (Palo Alto, Ca. USA) databases. Several human ESTs were identified and those with similarities greater than 50% selected for further analysis. One of these ESTs was T12251 previously shown to have 68% amino-
25 acid identity and 84% similarity over a region of 70 amino acids (15). All human ESTs from both databases were clustered to identify overlapping identical ESTs belonging to the same transcript. The GCG package (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wisconsin) and a program developed in
30 house termed ESTBlast (22) were used to build up these clusters. In total, forty-three ESTs derived from different tissue sources and both EST databases were clustered in ten groups named hVR2, hVR3, hVR1a, hVR1b, hVR1c, hVR1d, hVR1e, hVR1f, hVR1g and hVR1h. For each EST the tissue source was assigned according to the
35 annotations in the dbEST and Incyte databases. The shortest cluster

hVR1c also while having high homology (71% identity and 82% similarity over 65 residues) was closely related to the C-terminus of the rat protein sequence.

5   Cluster hVR1e shared a high homology (67% identity and 78% similarity for amino acids) with the 5' of the rat VR1 sequence but did not seem to have a potential start codon. It contained two Incyte ESTs (Clones Ids: 3801964 and 3802764) derived from the same tissue, bladder, and from the same patient. These two ESTs were
10   selected for further investigation since this cluster was the most 5', had high homology with rat VR1 and the bladder tissue could be contaminated with sensory neurons. Both cDNA clones were ordered but only 3801964 was received as 3802764 failed the recovering .procedure established at Incyte laboratories. The cDNA
15   insert was directionally cloned into the pINCY vector (Incyte).

The 3801964 clone was .grown using standard procedures and DNA was isolated using Qiagen columns. SP6 (5' ATTTAGGTGACACTATAG) and T7 (5'
20   TAATACGACTCACTATAGGG) primers flanking the cloning site of pINCY were used to sequence both ends. Plasmid DNA (0.6 pmol) was used with 10.0 pmol of each primer for the dye terminator reaction. The SP6 end corresponded to the *in silico* derived EST sequence (identical to 3801964). The T7 end did not have
25   homologies with VR1 nor did it possess a long open reading frame or a polyadenylation motif (data not shown). The size of the insert was determined by enzyme digestion of the DNA with the endonucleases NotI and EcoRI and calculated to be approximately 3kb (data not shown).
30
Plasmid DNA (50ng) was used to amplify the insert by Polymerase Chain Reaction (PCR) with T7 and SP6 as primers. The PCR conditions included an initial hot-start at 94°C for 2 minutes, followed by 35 cycles at 94°C for 45 seconds, 50°C for 45 seconds and 72°C
35   for 1 minute and terminated by 5 minutes at 72°C. The resulting

We formulated the hypothesis that both sequences (hVR1e and hVR3/1c) were part of a common transcript. If the human and rat VR1 were going to be similar, the 2 contigs should be separated by

5   a gap of approximately 275bp. Primers were designed on both sides of the gap to amplify mRNA from DRG and brain tissues in order to clone the gap (Figure 6). A smear was obtained with the sense primer (5' TCTACTTCGGTGAACTGCCC) and antisense (5' ACGGCAGGGAGTCATTCTTC). For specificity 50ng of the PCR

10 product were amplified with the nested sense (5' CTGCAGAACTCCTGGCAGA) and antisense (5' GTCACCACCGCTGTGGAAAA) primers. The 900bp nested amplicon was sequenced and shown to be identical to hVR1e at one end and hVR3/1c at the other end. The middle part of the PCR

15 product was homologous to the rat VR1 sequence. This region corresponded to 91 amino acids. When the sequences of hVR1e, hVR3/hVR1c and the internal amplicon are combined the total length of the Open Reading Frame (ORF) is 823 amino acids followed by a 3' untranslated sequence of 1120 bp. The human

20 amino acid sequence is 87% identical to the rat sequence over that part of the coding region. This sequence was termed hVR1 because of its high degree of identity with the rat VR1 sequence. It was a combination of the following clusters: HVR1c, hVR1e, hVR3.

25 Since no start codon was identified at the 5' end an additional strategy was designed to identify the full-length sequence. Two primers, sense (5' TCCTCTGGCTTCCAACCCGTT) and antisense (5' GAACTGGGCAGAAAGTGCCT) were designed to amplify a 150bp product from the first intron. A P1 Artificial Chromosome

30 (PAC) genomic clone (25) was isolated by PCR screening of a PAC library (Genome Systems, St Louis, Missouri). PAC DNA was recovered by using standard plasmid isolation protocol (26). An anti-sense primer was designed (5' CTGGAGTTAGGGTCTCCATCC) to sequence the genomic clone towards the potential 5' end of the

35 gene. An open reading frame with a starting codon was identified.

the hVR1 gene appears to be located on human chromosome 17 around marker SHGC-36073 (lod score=9.55).

In conclusion, we are confident that we have identified the full-length human orthologue of the rat VR1 gene because of the very high degree of homology with the rat transcript and a tissue distribution similar to that of rat VR1.

Identification and partial characterisation of additional human vanilloid receptors:

ESTs belonging to the remaining clusters were characterised by a combination of end sequencing, Bal-31 analysis and *in silico* cloning. The following Incyte clones were used during this process: -3324775 (hVR1a), -1725152 (hVR1b), -197476 (hVR1f), -3498406 (hVR1g), -1682513 (hVR1h), -1559496 (hVR2).

hVR2 was found to be a cluster of more than 40 ESTs derived from various tissues (and none from a DRG source). After *in silico* analysis and further sequencing of a particular Incyte clone, 1559496, two contiguous sequences were obtained: one located towards the 5' end and the other one more towards the 3' end. The former is 201 amino acid long while the latter has a size of 376 amino acids. A multiple comparison of these two contiguous sequences and the other vanilloid receptors is shown on figure 6. The 5' and 3' ends amino acid sequences of hVR2 have respectively 51% and 34% identity with the rat VR1 sequence.

A DNA probe from the coding region hVR2 was designed by enzyme restriction digest with Sty1 (New England Biolabs). A 438 bp fragment was obtained and used for radioactive hybridization on multi-Tissue northern blots (Clontech) according to the manufacturer's recommendations. As expected hVR2 is expressed in various tissues and a transcript of about 3.8 kb is detected by hybridization (figures 7 and 8).

shown by RT-PCR with the primer combination used to produce the probe that the gene is not expressed in DRG.

5   The 3' UTR sequence of hVR3 was used to design two primers to amplify a product of 360 bp: sense primer 5' ATGGCCACCAGCAGGGTTAC and antisense primer 5' TCTGCCAGGTTCCAGCTG. The G3 radiation hybrid panel from Stanford University (Research Genetics, Huntsville, Alabama) was screened by PCR. The positive lanes (eg: 11 lanes) and negative

10   patterns were analysed using the public web server at Stanford University (http://www-sghc.stanford.edu). After analysis the hVR3 gene appears to be located on human chromosome 12 around markers D12S177E (lod score=15) and D12S1893 (lod score=14).

15   The same primer combination was used to isolate a genomic PAC clone (Genome Systems. St Louis, Missouri) to be used to identify the complete 5' end of the hVR3 transcript.

We have concluded that hVR3 is an additional human gene member

20   of the vanilloid receptors family, but is not the orthologue of rVR1 because of its tissue distribution and homologies with rVR1.

hVR1g and hVR1b collapsed in a single contiguous sequence. Sequence analysis has shown that both cDNAs are likely to be

25   chimeric. The 5' end has weak similarities with the rat VR1 gene but the 3' end is identical to a DNA binding protein.

Screening for compounds which exhibit hVR modulating activity

30   Mammalian cells, such as Hek293, CHO and HeLa cells over-expressing the VR receptor of choice are generated for use in the assay. 96 and 384 well plate, high throughput screens (HTS) are employed using fluorescence based calcium indicator molecules, including but not limited to dyes such as Fura-2, Fura-Red, Fluo 3

35   and Fluo 4 (Molecular Probes). Secondary screening involves

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10.   The nucleotide sequence according to any one of claims 6 to 9 which is a cDNA sequence.

11.   The nucleotide sequence according to claim 6 as shown in Figure 1 or Figure 9.

12.   An expression vector comprising a nucleotide sequence according to any one of claims 6 to 11, which is capable of expressing an hVR protein.

13.   The expression vector according to claim 12 capable of expressing a protein selected from hVR1, hVR2 or hVR3.

14.   The expression vector according to claim 12 capable of expressing hVR1.

15.   A stable cell line comprising an expression vector according to any one of claims 12 to 14.

16.   The cell line according to claim 15 which is a modified HEK293, C40 or HeLa cell line.

17.   An antibody specific for a protein as claimed in any one of claims 1 to 5.

18.   A method for identification of a compound which exhibits hVR modulating activity comprising contacting an hVR protein according to any of claims 1 to 5 with a test compound and detecting modulating activity or inactivity.

19.   A compound which modulates hVR activity, identifiable by a method according to claim 18, excluding the compounds capsaicin, resiniferatoxin and capsazepine.

Figure 1: hVR1 nucleotide sequence

```
   1  CGTGGTGGCT GCTGCAGGTT GCACACTGGG CCACAGAGGA TCCAGCAAGG
  51  ATGAAGAAAT GGAGCAGCAC AGACTTGGGG GCAGCTGCGG ACCCACTCCA
 101  AAAGGACACC TGCCCAGACC CCCTGGATGG AGACCCTAAC TCCAGGCCAC
 151  CTCCAGCCAA GCCCCAGCTC TCCACGGCCA AGAGCCGCAC CCGGCTCTTT
 201  GGGAAGGGTG ACTCGGAGGA GGCTTTCCCG GTGGATTGCC CTCACGAGGA
 251  AGGTGAGCTG GACTCCTGCC CGACCATCAC AGTCAGCCCT GTTATCACCA
 301  TCCAGAGGCC AGGAGACGGC CCCACCGGTG CCAGGCTGCT GTCCCAGGAC
 351  TCTGTCGCCG CCAGCACCGA GAAGACCCTC AGGCTCTATG ATCGCAGGAG
 401  TATCTTTGAA GCCGTTGCTC AGAATAACTG CCAGGATCTG GAGAGCCTGC
 451  TGCTCTTCCT GCAGAAGAGC AAGAAGCACC TCACAGACAA CGAGTTCAAA
 501  GACCCTGAGA CAGGGAAGAC CTGTCTGCTG AAAGCCATGC TCAACCTGCA
 551  CGACGGACAG AACACCACCA TCCCCCTGCT CCTGGAGATC GCGCGGCAAA
 601  CGGACAGCCT GAAGGAGCTT GTCAACGCCA GCTACACGGA CAGCTACTAC
 651  AAGGGCCAGA CAGCACTGCA CATCGCCATC GAGAGACGCA ACATGGCCCT
 701  GGTGACCCTC CTGGTGGAGA ACGGAGCAGA CGTCCAGGCT GCGGCCCATG
 751  GGGACTTCTT TAAGAAAACC AAAGGGCGGC CTGGATTCTA CTTCGGTGAA
 801  CTGCCCCTGT CCCTGGCCGC GTGCACCAAC CAGCTGGGCA TCGTGAAGTT
 851  CCTGCTGCAG AACTCCTGGC AGACGGCCGA CATCAGCGCC AGGGACTCGG
 901  TGGGCAACAC GGTGCTGCAC GCCCTGGTGG AGGTGGCCGA CAACACGGCC
 951  GACAACaCGA AGTTTGTgAC gAGCATGtaC AaTgAGATTC TGATCCTGGG
1001  GGCCAAACTG CAcCCGACGC TgAAGCTgGA GGAGCTCACC aACaAGAAGG
1051  GAATGACGCC GCTGGCTCTG GCAGCTGGGA CCgGGAAGAT CGGGGTCTTG
1101  GCCTATATTC TCCAGCGGGA GATCCAGGAG CCCGAGTGCA GGCACCTGTC
1151  CAGGAAGTTC ACCGAGTGgg cCTACGGGCC CGTGCACTCC TCGCTGTACG
1201  ACCTGTCCTG CATCGACACC TGCGAGAAGA ACTCGGTGCT GGAGGTGATC
1251  GCCTACAGCA GCAGCGAGAC CCCTAATCGC CACGACATGC TCTTGGTGGA
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1301  GCCGCTGAAC CGACTCCTGC AGGACAAGTG GGACAGATTC GTCAAGCGCA
1351  TCTTCTACTT CAACTTCCTG GTCTACTGCC TGTACATGAT CATCTTCACC
1401  ATGGCTGCCT ACTACAGGCC CGTGGATGGC TTGCCTCCCT TTAAGATGGA
1451  AAAAACTGGA GACTATTTCC GAGTTACTGG AGAGATCCTG TCTGTGTTAG
1501  GAGGAGTCTA CTTCTTTTTC CGAGGGATTC AGTATTTCCT GCAGAGGCGG
1551  CCGTCGATGA AGACCCTGTT TGTGGACAGC TACAGTGAGA TGCTTTTCTT
1601  TCTGCAGTCA CTGTTCATGC TGGCCACCGT GGTGCTGTAC TTCAGCCACC
1651  TCAAGGAGTA TGTGGCTTCC ATGGTATTCT CCCTGGCCTT GGGCTGGACC
1701  AACATGCTCT ACTACACCCG CGGTTTCCAG CAGATGGGCA TCTATGCCGT
1751  CATGATAGAG AAGATGATCC TGAGAGACCT GTGCCGTTTC ATGTTTGTCT
1801  ACATCGTCTT CTTGTTCGGG TTTTCCACAG CGGTGGTGAC GCTGATTGAA
1851  GACGGGAAGA ATGACTCCCT GCCGTCTGAG TCCACGTCGC ACAGGTGGCG
1901  GGGGCCTGCC TGCAGGCCCC CCGATAGCTC CTACAACAGC CTGTACTCCA
1951  CCTGCCTGGA GCTGTTCAAG TTCACCATCG GCATGGGCGA CCTGGAGTTC
2001  ACTGAGAACT ATGACTTCAA GGCTGTCTTC ATCATCCTGC TGCTGGCCTA
2051  TGTAATTCTC ACCTACATCC TCCTGCTCAA CATGCTCATC GCCCTCATGG
2101  GTGAGACTGT CAACAAGATC GCACAGGAGA GCAAGAACAT CTGGAAGCTG
2151  CAGAGAGCCA TCACCATCCT GGACACGGAG AAGAGCTTCC TTAAGTGCAT
2201  GAGGAAGGCC TTCCGCTCAG GCAAGCTGCT GCAGGTGGGG TACACACCTG
2251  ATGGCAAGGA CGACTACCGG TGGTGCTTCA GGGTGGACGA GGTGAACTGG
2301  ACCACCTGGA ACACCAACGT GGGCATCATC AACGAAGACC CGGGCAACTG
2351  TGAkGGCGTC AAGCGCACCC TGAGCTTCTC CCTGCGGTCA AGCAGAGTTT
2401  CAGGCAGACA CTGGAAGAAC TTTGCCCTGG TCCCCCTTTT AAGAGAGGCA
2451  AGTGCTCGAG ATAGGCAGTC TGCTCAGCCC GAGGAAGTTT ATCTGCGACA
2501  GTTTTCAGGG TCTCTGAAGC CAGAGGACGC TGAGGTCTTC AAGAGTCCTG
2551  CCGCTTCCGG GGAGAAGTGA GGACGTCACG CAGACAGCAC TGTCAACACT
2601  GGGCCTTAGG AGACCCCGTT GCCACGGGGX XCTGCTGAGG AACACCAGT
2651  GCTCTGTCAG CAGCCTGGCC TGGTCTGTGC CTGCCCAGCA TGTTCCCAAA
2701  TCTGTGCTGG ACAAGCTGTG GGAAGCGTTC TTGGAAGCAT GGGGAGTGAT
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2751 GTACATCCAA CCGTCACTGT CCCCAAGTGA ATCTCCTAAC AGACTTTCAG

2801 GTTTTTACTC ACTTTACTAA ACAGTTTGGA TGGTCAGTCT CTACTGGGAC

2851 ATGTTAGGCC CTTGTTTTCT TTGATTTTAT TCTTTTCTGT GAGACAGAGT

2901 TCACTCTTGT TGCCCAGGCT GGAGTGCAGT GGTGTGATCT TGGCTCACTG

2951 CAACCTCTGC TCCCGGGTTC AAGCGATTCT TCTGCTTCAG TCTCCCAAGT

3001 AGCTTGGATT ACAGGTGAGC ACTACCACGC CCGGCTAATT TTTGTATTTT

3051 TAATAGAGAC GGGGTTTCAC CATGTTGGCC AGGCTGGTCT CGAACTCTTG

3101 ACCTCAGGTG ATCTGCCCGC CTTGGCCTCC CAAAGTGCTG GGATTACAGG

3151 TGTGAGCCGC TGCGCTCGGC CTTCTTTGAT TTTATATTAT TAGGAGCAAA

3201 AGTAAATGAA GCCCAGGAAA ACACCTTTGG GAACAAACTC TTCCTTTGAT

3251 GGAAAATGCA GAGGCCCTTC CTCTCTGTGC CGTGCTTGCT CCTCTTACCT

3301 GCCCGGGTGG TTTGGGGGTG TTGGTGTTTC CTCCCTGGAG AAGATGGGGG

3351 AGGCTGTCCC ACTCCCAGCT CTGGCAGAAT CAAGCTGTTG CAGCAGTGCC

3401 TTCTTCATCC TTCCTTACGA TCAATCACAG TCTCCAGAAG ATCAGCTCAA

3451 TTGCTGTGCA GGTTAAAACT ACAGAACCAC ATCCCAAAGG TACCTGGTAA

3501 GAATGTTTGA AAGATCTTCC ATTTCTAGGA ACCCCAGTCC TGCTTCTCCG

3551 CAATGGCACA TGCTTCCACT CCATCCATAC TGGCATCCTC AAATAAACAG

3601 ATATGTATAC AAAAAAAAAA AAAAAAAAAA AAAAAAAAA A
```

Figure 2: Nucleotide and amino acid sequence of hVR1

```
 -50  cgtggtggctgctgcaggttgcacactgggccacagaggatccagcaaggATGAAGAAAT  10
                                                         M   K   K   W 4


  11  GGAGCAGCACAGACTTGGGGGCAGCTGCGGACCCACTCCAAAAGGACACCTGCCCAGACC  70
   5   S   S   T   D   L   G   A   A   A   D   P   L   Q   K   D   T   C   P   D   P 24


  71  CCCTGGATGGAGACCCTAACTCCAGGCCACCTCCAGCCAAGCCCCAGCTCTCCACGGCCA 130
  25   L   D   G   D   P   N   S   R   P   P   P   A   K   P   Q   L   S   T   A   K 44


 131  AGAGCCGCACCCGGCTCTTTGGGAAGGGTGACTCGGAGGAGGCTTTCCCGGTGGATTGCC 190
  45   S   R   T   R   L   F   G   K   G   D   S   E   E   A   F   P   V   D   C   P 64


 191  CTCACGAGGAAGGTGAGCTGGACTCCTGCCCGACCATCACAGTCAGCCCTGTTATCACCA 250
  65   H   E   E   G   E   L   D   S   C   P   T   I   T   V   S   P   V   I   T   I 84


 251  TCCAGAGGCCAGGAGACGGCCCCACCGGTGCCAGGCTGCTGTCCCAGGACTCTGTCGCCG 310
  85   Q   R   P   G   D   G   P   T   G   A   R   L   L   S   Q   D   S   V   A   A 104


 311  CCAGCACCGAGAAGACCCTCAGGCTCTATGATCGCAGGAGTATCTTTGAAGCCGTTGCTC 370
 105   S   T   E   K   T   L   R   L   Y   D   R   R   S   I   F   E   A   V   A   Q 124


 371  AGAATAACTGCCAGGATCTGGAGAGCCTGCTGCTCTTCCTGCAGAAGAGCAAGAAGCACC 430
 125   N   N   C   Q   D   L   E   S   L   L   L   F   L   Q   K   S   K   K   H   L 144


 431  TCACAGACAACGAGTTCAAAGACCCTGAGACAGGGAAGACCTGTCTGCTGAAAGCCATGC 490
 145   T   D   N   E   F   K   D   P   E   T   G   K   T   C   L   L   K   A   M   L 164


 491  TCAACCTGGACGACGGACAGAACACCACGATCCCCCTGCTCGTGGAGATCGCGCGGCAAA 550
 165   N   L   H   D   G   Q   N   T   T   I   P   L   L   L   E   I   A   R   Q   T 184


 551  CGGACAGCCTGAAGGAGTTGTCAACGCCAGCTACACGGACAGCTACTACAAGGGCCAGA 610
 185   D   S   L   K   E   L   V   N   A   S   Y   T   D   S   Y   Y   K   G   Q   T 204


 611  CAGCACTGCACATCGCCATCGAGAGACGCAACATGGCCCTGGTGACCCTCCTGGTGGAGA 670
 205   A   L   H   I   A   I   E   R   R   N   M   A   L   V   T   L   L   V   E   N 224


 671  ACGGAGCAGACGTCCAGGCTGCGGCCCATGGGGACTTCTTTAAGAAAACCAAAGGGCGGC 730
 225   G   A   D   V   Q   A   A   A   H   G   D   F   F   K   K   T   K   G   R   P 244


 731  CTGGATTCTACTTCGGTGAACTGCCCCTGTCCCTGGCCGCGTGCACCAACCAGCTGGGCA 790
 245   G   F   Y   F   G   E   L   P   L   S   L   A   A   C   T   N   Q   L   G   I 264


 791  TCGTGAAgTTCCTGCTGCAGAACTCCTGGCAGACGGCCGACATCAGCGCCAGGGACTCGG 850
 265   V   K   F   L   L   Q   N   S   W   Q   T   A   D   I   S   A   R   D   S   V 284


 851  TGGGCAACACGGTGCTGCACGCCCTGGTGGAGGTGGCCGACAACaCGGCCGACAACaCGA 910
 285   G   N   T   V   L   H   A   L   V   E   V   A   D   N   T   A   D   N   T   K 304


 911  AGTTTGTgACgAGCATGtaCAaTgAGATTCTGATCCTGGGGGCCAAACTGCAcCCGACGC 970
 305   F   V   T   S   M   Y   N   E   I   L   I   L   G   A   K   L   H   P   T   L 324


 971  TgAAGCTgGAGGAGCTCACCaACaAGAAGGGAATGACGCCGCTGGCTCTGGCAGCTGGGA 1030
 325   K   L   E   E   L   T   N   K   K   G   M   T   P   L   A   L   A   A   G   T 344


1031  CCgGGAAGATCGGGGTCTTGGCCTATATTCTCCAGCGGGAGATCCAGGAGCCCGAGTGCA 1090
 345   G   K   I   G   V   L   A   Y   I   L   Q   R   E   I   Q   E   P   E   C   R 364


1091  GGCACCTGTCCAGGAAGTTCACCGAGTGggcCTACGGGCCCGTGCACTCCTCGCTGTACG 1150
 365   H   L   S   R   K   F   T   E   W   A   Y   G   P   V   H   S   S   L   Y   D 384


1151  ACCTGTCCTGCATCGACACCTGCGAGAAGAACTCGGTGCTGGAGGTGATCGCCTACAGCA 1210
 385   L   S   C   I   D   T   C   E   K   N   S   V   L   E   V   I   A   Y   S   S 404


1211  GCAGCGAGACCCCTAATCGCCACGACATGCTCTTGGTGGAGCCGCTGAACCGACTCCTGC 1270
 405   S   E   T   P   N   R   H   D   M   L   L   V   E   P   L   N   R   L   L   Q 424


1271  AGGACAAGTGGGACAGATTCGTCAAGCGCATCTTCTACTTCAACTTCCTGGTCTACTGCC 1330
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425      D  K  W  D  R  F  V  K  R  I  F  Y  F  N  F  L  V  Y  C  L 444

1331 TGTACATGATCATCTTCACCATGGCTGCCTACTACAGGCCCGTGGATGGCTTGCCTCCCT 1390
445      Y  M  I  I  F  T  M  A  A  Y  Y  R  P  V  D  G  L  P  P  F 464

1391 TTAAGATGGAAAAAACTGGAGACTATTTCCGAGTTACTGGAGAGATCCTGTCTGTGTTAG 1450
465      K  M  E  K  T  G  D  Y  F  R  V  T  G  E  I  L  S  V  L  G 484

1451 GAGGAGTCTACTTCTTTTTCCGAGGGATTCAGTATTTCCTGCAGAGGCGGCCGTCGATGA 1510
485      G  V  Y  F  F  F  R  G  I  Q  Y  F  L  Q  R  R  P  S  M  K 504

1511 AGACCCTGTTTGTGGACAGCTACAGTGAGATGCTTTTCTTTCTGCAGTCACTGTTCATGC 1570
505      T  L  F  V  D  S  Y  S  E  M  L  F  F  L  Q  S  L  F  M  L 524

1571 TGGCCACCGTGGTGCTGTACTTCAGCCACCTCAAGGAGTATGTGGCTTCCATGGTATTCT 1630
525      A  T  V  V  L  Y  F  S  H  L  K  E  Y  V  A  S  M  V  F  S 544

1631 CCCTGGCCTTGGGCTGGACCAACATGCTCTACTACACCCGCGGTTTCCAGCAGATGGGCA 1690
545      L  A  L  G  W  T  N  M  L  Y  Y  T  R  G  F  Q  Q  M  G  I 564

1691 TCTATGCCGTCATGATAGAGAAGATGATCCTGAGAGACCTGTGCCGTTTCATGTTTGTCT 1750
565      Y  A  V  M  I  E  K  M  I  L  R  D  L  C  R  F  M  F  V  Y 584

1751 ACATCGTCTTCTTGTTCGGGTTTTCCACAGCGGTGGTGACGCTGATTGAAGACGGGAAGA 1810
585      I  V  F  L  F  G  F  S  T  A  V  V  T  L  I  E  D  G  K  N 604

1811 ATGACTCCCTGCCGTCTGAGTCCACGTCGCACAGGTGGCGGGGGCCTGCCTGCAGGCCCC 1870
605      D  S  L  P  S  E  S  T  S  H  R  W  R  G  P  A  C  R  P  P 624

1871 CCGATAGCTCCTACAACAGCCTGTACTCCACCTGCCTGGAGCTGTTCAAGTTCACCATCG 1930
625      D  S  S  Y  N  S  L  Y  S  T  C  L  E  L  F  K  F  T  I  G 644

1931 GCATGGGCGACCTGGAGTTCACTGAGAACTATGACTTCAAGGCTGTCTTCATCATCCTGC 1990
645      M  G  D  L  E  F  T  E  N  Y  D  F  K  A  V  F  I  I  L  L 664

1991 TGCTGGCCTATGTAATTCTCACCTACATCCTCCTGCTCAACATGCTCATCGCCCTCATGG 2050
665      L  A  Y  V  I  L  T  Y  I  L  L  L  N  M  L  I  A  L  M  G 684

2051 GTGAGACTGTCAACAAGATCGCACAGGAGAGCAAGAACATCTGGAAGCTGCAGAGAGCCA 2110
685      E  T  V  N  K  I  A  Q  E  S  K  N  I  W  K  L  Q  R  A  I 704

2111 TCACCATCCTGGACACGGAGAAGAGCTTCCTTAAGTGCATGAGGAAGGCCTTCCGCTCAG 2170
705      T  I  L  D  T  E  K  S  F  L  K  C  M  R  K  A  F  R  S  G 724

2171 GCAAGCTGCTGCAGGTGGGGTACACACCTGATGGCAAGGACGACTACCGGTGGTGCTTCA 2230
725      K  L  L  Q  V  G  Y  T  P  D  G  K  D  D  Y  R  W  C  F  R 744

2231 GGGTGGACGAGGTGAACTGGACCACCTGGAACACCAACGTGGGCATCATCAACGAAGACC 2290
745      V  D  E  V  N  W  T  T  W  N  T  N  V  G  I  I  N  E  D  P 764

2291 CGGGCAACTGTGAkGGCGTCAAGCGCACCCTGAGCTTCTCCCTGCGGTCAAGCAGAGTTT 2350
765      G  N  C  ?  G  V  K  R  T  L  S  F  S  L  R  S  S  R  V  S 784

2351 CAGGCAGACACTGGAAGAACTTTGCCCTGGTCCCCCTTTTAAGAGAGGCAAGTGCTCGAG 2410
785      G  R  H  W  K  N  F  A  L  V  P  L  L  R  E  A  S  A  R  D 804

2411 ATAGGCAGTCTGCTCAGCCCGAGGAAGTTTATCTGCGACAGTTTTCAGGGTCTCTGAAGC 2470
805      R  Q  S  A  Q  P  E  E  V  Y  L  R  Q  F  S  G  S  L  K  P 824

2471 CAGAGGACGCTGAGGTCTTCAAGAGTCCTGCCGCTTCCGGGGAGAAGTGAggacgtcacg 2530
825      E  D  A  E  V  F  K  S  P  A  A  S  G  E  K  *              840

2531 cagacagcactgtcaacactgggccttaggagaccccgttgccacggggxxctgctgagg 2590

2591 gaacaccagtgctctgtcagcagcctggcctggtctgtgcctgcccagcatgttcccaaa 2650

2652 tctgtgctggacaagctgtgggaagcgttcttggaagcatggggagtgatgtacatccaa 2710

2711 ccgtcactgtccccaagtgaatctcctaacagactttcaggttttttactcactttactaa 2770

2771 acagtttggatggtcagtctctactgggacatgttaggcccttgttttctttgatttttat 2830
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2831 tcttttctgtgagacagagttcactcttgttgcccaggctggagtgcagtggtgtgatct 2890

2891 tggctcactgcaacctctgctcccgggttcaagcgattcttctgcttcagtctcccaagt 2950

2951 agcttggattacaggtgagcactaccacgcccggctaatttttgtatttttaatagagac 3010

3011 ggggtttcaccatgttggccaggctggtctcgaactcttgacctcaggtgatctgcccgc 3070

3071 cttggcctcccaaagtgctgggattacaggtgtgagccgctgcgctcggccttctttgat 3130

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3191 ttcctttgatggaaaatgcagaggcccttcctctctgtgccgtgcttgctcctcttacct 3250

3251 gcccgggtggtttgggggtgttggtgtttcctccctggagaagatgggggaggctgtccc 3310

3311 actcccagctctggcagaatcaagctgttgcagcagtgccttcttcatccttccttacga 3370

3371 tcaatcacagtctccagaagatcagctcaattgctgtgcaggttaaaactacagaaccac 3430

3431 atcccaaaggtacctggtaagaatgtttgaaagatcttccatttctaggaaccccagtcc 3490

3491 tgcttctccgcaatggcacatgcttccactccatccatactggcatcctcaaataaacag 3550

3551 atatgtatacaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa 3591
```

Figure 3: Amino acid sequence of hVR1

```
  1    MKKWSSTDLG AAADPLQKDT CPDPLDGDPN SRPPPAKPQL STAKSRTRLF

 51    GKGDSEEAFP VDCPHEEGEL DSCPTITVSP VITIQRPGDG PTGARLLSQD

101    SVAASTEKTL RLYDRRSIFE AVAQNNCQDL ESLLLFLQKS KKHLTDNEFK

151    DPETGKTCLL KAMLNLHDGQ NTTIPLLLEI ARQTDSLKEL VNASYTDSYY

201    [                                        ]GDFFKKT KGRPGFY[  ]

251    [                                ]RDSVGNTVLH ALVEVADNTA

301    DNTKFVTSMY NEILILGAKL HPTLKLEELT NK[                    ]

351    [                    ]LSRKF TEWAYGPVHS SLYDLSCIDT CEKNSVLEVI

401    AYSSSETPNR HDMLLVEPLN RLLQDKWDRF VKR███████████████████

451    ████RPVDG LPPFKMEKTG DYFRVTGEI██████████████FLQRR

501    PSMKTLFVD██████████████████HLKEYVAS MV██████

551    ██████████████E KMILRD██████████████TLIE

601    DGKNDSLPSE STSHRWRGPA CRPPDSSYNS LYSTCLELFK FTIGMGDLEF

651    TENYD████████████████████ETVNKI AQESKNIWKL

701    QRAITILDTE KSFLKCMRKA FRSGKLLQVG YTPDGKDDYR WCFRVDEVNW

751    TTWNTNVGII NEDPGNCXGV KRTLSFSLRS SRVSGRHWKN FALVPLLREA

801    SARDRQSAQP EEVYLRQFSG SLKPEDAEVF KSPAASGEK*
```

Key

T/S predicted phosphorylation sites

███ Transmembrane domains

[ ] Ankyrin binding domains

Figure 4: Comparison of the amino acid sequences of the rat (rVR1) and human (hVR1) vanilloid proteins.

Figure 5: Slot Blot hybridisation with hVR1 probe

Well

1A hDRG	1B hDRG
2A rDRG	
3A rat Spinal cord	3B hSpinal Cord

4B Water
5B Incyte clone 1427927
6B 900bp Amplicon from Brain cDNA
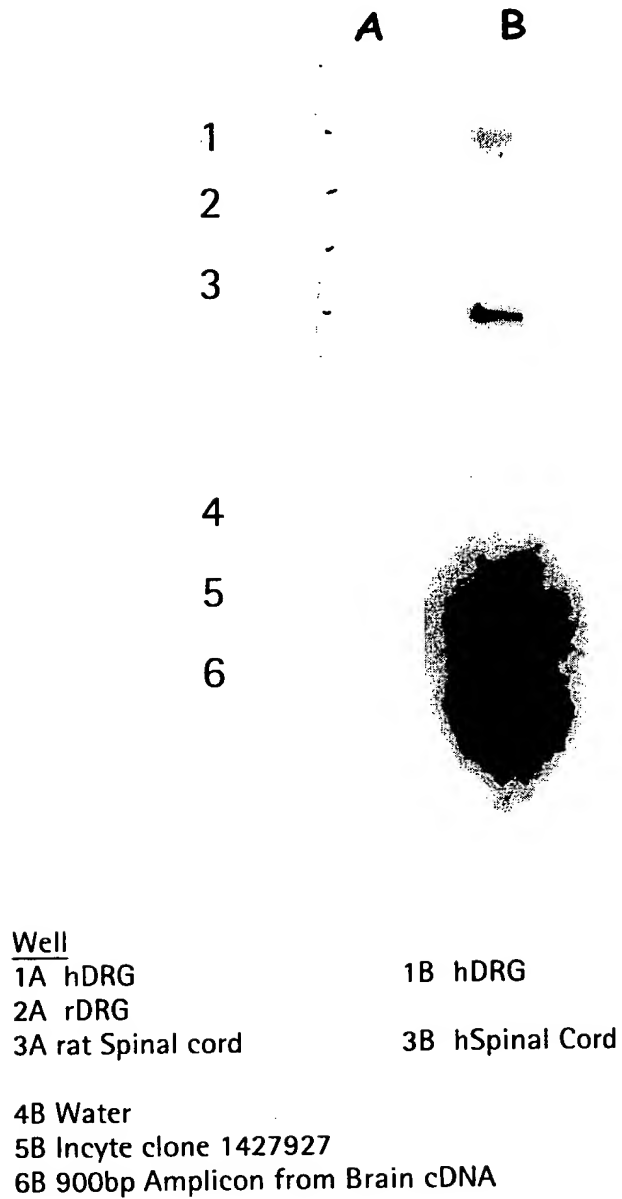
Figure 6: Multiple comparison of the amino acid sequ nces of rat VR1 and the human vanilloid receptors: hVR1, the 5' and 3' ends of hVR2 and the 3' end of hVR3.

igure 7

```
   1   ACCGGCGAAC GTGCNAGAAG GAAGGAAGAA AGCAAAGAAC GGCGTAGGGC

  51   GCTGGCAAGT GTAGCGGTCA CGCTGCGCGT AACCACCACA CCCGCCGCGC

 101   TTAATGCGCC GCTACAGGGC GCGTCCCATT CGCCATTCAG GCTGCGCAAC

 151   TGTTGGGAAG GGCGATCGGT GCGGGCCTCT TCGCTATTAC GCCAGCTGGC

 201   GAAAGGGGGA TGTGCTGCAA GGCGATTAAG TTGGGTAACG CCAGGGTTTT

 251   CCCAGTCACG ACGTTGTAAA ACGACGGCCA GTGAATTGAA TTTAGGTGAC

 301   ACTATAGAAG AGCTATGACG TCGCATGCAC GCGTACGTAA GCTCGGAATT

 351   CGGCTCGAGC CCACACCAGC CCGCCAGCCT GCAGGCCACT GACTCCCAGG

 401   GCAACACAGT CCTGCATGCC CTAGTGATGA TCTCGGACAA CTCAGCTGAG

 451   AACATTGCAC TGGTGACCAG CATGTATGAT GGGCTCCTCC AAGCTGGGGC

 501   CCGCCTCTGC CCTACCGTGC AGCTTGAGGA CATCCGCAAC CTGCAGGATC

 551   TCACGCCTCT GAAGCTGGCC GCCAAGGAGG GCAAGATCGA GATTTTCAGG

 601   CACATCCTGC AGCGGGAGTT TTCAGGACTG AGCCACCTTT CCCGAAAGTT

 651   CACCGAGTGG TGCTATGGGC CTGTCCGGGT GTCGCTGTAT GACCTGGCTT

 701   CTGTGGACAG CTGTGAGGAG AACTCAGTGC TGGAGATCAT TGCCTTTCAT

 751   TGCAAGAGCC CGCACCGACA CCGAATGGTC GTTTTGGAGC CCCTGAACAA

 801   ACTGCTGCAG GCGAAATGGG ATCTGCTCAT CCCCAAGTTC TTCTTAAACT

 851   TCCTGTGTAA TCTGATCTAC ATGTTCATCT TCACCGCTGT TGCCTACCAT

 901   CAGCCTACCC TGAAGAAGCA GGCCGCCCCT CACCTGAAAG CGGAGGTTGG

 951   AAACTCCATG CTGCTGACGG GCCACATCCT TATCCTGCTA GGGGGGATCT

1001   ACCTCCTCGT GGGCCAGCTG TGGTACTTCT GGCGGCGCCA CGTGTTCATC

1051   TGGATCTCGT TCATAGACAG CTACTTTGAA ATCCTCTTCC TGTTCCAGGC

1101   CCTGCTCACA GTGGTGTCCC AGGTGCTGTG TTTCCTGGCC ATCGAGTGGT

1151   ACCTGCCCCT GCTTGTGTCT GCGCTGGTGC TGGGCTGGCT GAACCTGCTT

1201   TACTATACAC GTGGCTTCCA GCACACAGGC ATCTACAGTG TCATGATCCA

1251   GAAGGTCATC CTGCGGGACC TGCTGCGCTT CCTTCTGATC TACTTAGTCT

1301   TCCTTTTCGG CTTCGCTGTA GCCCTGGTGA GCCTGAGCCA GGAGGCTTGG
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1351   CGCCCCGAAG CTCCTACAGG CCCCAATGCC ACAGAGTCAG TGCAGCCCAT

1401   GGAGGGACAG GAGGACGAGG GCAACGGGGC CCAGTACAGG GGTATCCTGG

1451   AAGCCTCCTT GGAGCTCTTC AAATTCACCA TCGGCATGGG CGAGCTGGCC

1501   TTCCAGGAGC AGCTGCACTT CCGCGGCATG GTGCTGCTGC TGCTGCTGGC

1551   CTACGTGCTG CTCACCTACA TCCTGCTGCT CAACATGCTC ATCGCCCTCA

1601   TGAGCGAGAC CGTCAACAGT GTCGCCACTG ACAGCTGGAG CATCTGGAAG

1651   CTGCAGAAAG CCATCTCTGT CCTGGAGATG GAGAATGGCT ATTGGTGGTG

1701   CAGGAAGAAG CAGCGGGCAG GTGTGATGCT GACCGTTGGC ACTAAGCCAG

1751   ATGGCAGCCC CGATGAGCGC TGGTGCTTCA GGGTGGAGGA GGTGAACTGG

1801   GCTTCATGGG AGCAGACGCT GCCTACGCTG TGTGAGGACC CGTCAGGGGC

1851   AGGTGTCCCT CGTGAGTAGC CTGGAACTCT CGAGAACCCT GTCCTGGCTT

1901   CCCCTCCCAA GGAGGATGAG GATGGTGCCT CTGAGGAAAA CTATGTGCCC

1951   GTCCAGCTCC TCAGTCCAAC TGATGGCCCA GATGCAGCAG GAGGCCAGAG

2001   GACAGAGCAG AGGATCTTTC CAACCACATC TGCTGGCTCT GGGGTCCCAG

2051   TGAATTCTGG TGGCAAATAT ATATTTCAC TAACTAAAAA AAAAAAAAAA
```

igure 8

```
   1  GGGAAGAATC CCCATCnATG GCAGCTTCCA TGGGTGGCAA GTCCCCAGCA

  51  TCCAAGGGCT GCCTCTGAGn GTCACCCACC CCCACCTGAG ACCTTAGTGG

 101  CTAGAATnnG GAnGGnTGGn GGTGGAnCCT nAnTCGCAGC AGGGTGTGTC

 151  CAGATGGTCA GTCTCTGGTG GCTAGCCTGT CCTGACAGGG GAGAGTTAAG

 201  CTCCCGtTCT CCACCGTGCC GGCTGGCaGG TGGGCTGAGG GTGACCGAGA

 251  GACCAGAACC TGCTTGCTGG AGCTTAGTGC TCAGAGCTGG GGAGGGAGGT

 301  TCCGCCGCTC CTCTGCTGTC AGCGCCGGCA GCCCCTCCCG GCTTCACTTC

 351  CTCCCGCAGC CCCTGCTACT GAGAAGCTCC GGGATCCCAG CAGCCGCCAC

 401  GCCCTGGCCT CAGCCTGCGG GGCTCCAGTC AGGCCAACAC CGACGCGCAn

 451  CTGGnGAGGA AGACAGGACC CTTGACATCT CCATCTGCAC AGAGGTCCTG

 501  GCTGGACCGA GCAGCCTCCT CCTCCTAGGA TGACCTCACC CTCCAGCTCT

 551  CCAGTTTTCA GGTTGGAGAC ATTAGATGGA GGCCAAGAAG ATGGCTCTGA

 601  GGCGGACAGA GGAAAGCTGG ATTTTGGGAG CGGGCTGCCT CCCATGGAGT

 651  CACAGTTCCA GGGCGAGGAC CGGAAATTCG CCCCTCAGAT AAGAGTCAAC

 701  CTCAACTACC GAAAGGGAAC AGGTGCCAGT CAGCCGGATC CAAACCGATT

 751  TGACCGAGAT CGGCTCTTCA ATGCGGTCTC CCGGGGTGTC CCCGAGGATC

 801  TGGCTGGACT TCCAGAGTAC CTGAGCAAGA CCAGCAAGTA CCTCACCGAC

 851  TCGGAATACA CAGAGGGCTC CACAGGTAAG ACGTGCCTGA TGAAGGCTGT

 901  GCTGAACCTT AAGGACGGGG TCAATGCCTG CATTCTGCCA CTGCTGCAGA

 951  TCGACmGGGA CTCTGGCAAT CCTCAGCCCC TGGTAAATGC CCAGTGCACA

1001  GATGACTATT ACCGAGGCCA CAGCGCTCTG CACATCGCCA TTGAGAAGAG

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1101  CCCGGGCCTG CGGCGCAAGC TTATTCCCTT TA
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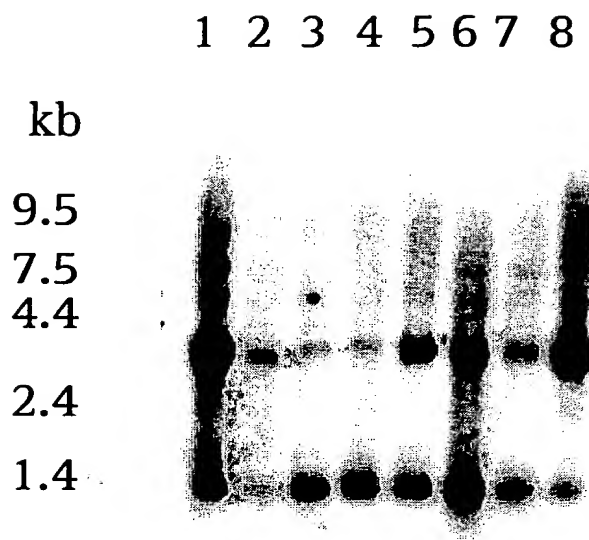
Figure 9

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   1  cTGCACCTGT CGCTGGCTGC CTGCACnAAC CAGnnCCACA TTGTCAACTA
  51  CCTGACGGAG AACCCCCACA AGAAGGCGGA CATGCGGCGC CAGGACTCGC
 101  GAGGCAACAC AGTGCTGCAT GCGCTGGTGG CCATTGCTGA CAACACCCGT
 151  GAGAACACCA AGTTTGTTAC CAAGATGTAC GACCTGCTGC TGCTCAAGTG
 201  TGCnCGCCTC TTCCCCGACA GCAACCTGGA nGCCGTGCTC AACAACGACG
 251  GCCTCTCGCC CCTCATGATG GCTGCCAAGA CGGGCAAGAT TGGGAwCyTT
 301  CAGCACATCA TCCGGCGGGA GGTGACGGAT GAGGACACAC GGCACCTGTC
 351  CCGCAAGTTC AAGGACTGGG CCTATGGGCC AGTGTATTCC TCGCTTTATG
 401  ACCTCTCCTC CCTGGACACG TGTGGGGAAG AGGCCTCCGT GCTGGAGATC
 451  CTGGTGTACA ACAGCAAGAT TGAGAACCGC CACGAGATGC TGGCTGTGGA
 501  GCCCATCAAT GAACTGCTGC GGGACAAGTG GCGCAAgTTC GGGGCCGTCT
 551  CCTTCTACAT CAACGTGGTC TCCTACCTGT GTGCCATGGT CATCTTCACT
 601  CTCACCGCCT ACTACCAGCC GCTGGAGGGC ACACCGCCGT ACCCTTACCG
 651  CACCACGGTG GACTACCTGC GGCTGGCTGG CGAGGTCATT ACGCTCTTCA
 701  CTGGGGTCCT GTTCTTCTTC ACCAACATCA AAGACTTGTT CATGAAGAAA
 751  TGCCCTGGAG TGAATTCTCT CTTCATTGAT GGCTCCTTCC AGCTGCTCTA
 801  CTTCATCTAC TCTGTCCTGG TGATCGTCTC AGCAGCCCTC TACCTGGCAG
 851  GGATCGAGGC CTACCTGGCC GTGATGGTCT TTGCCCTGGT CCTGGGCTGG
 901  ATGAATGCCC TTTACTTCAC CCGTGGGCTG AAGCTgacgg ggacctataG
 951  CATCATGATC CAGAAGATTC TCTTCAAGGA CCTTTTCCGA TTCCTGCTCG
1001  TCTACTTGCT CTTCATGATC GGCTACGCTT CAGCCCTGGT CTCCCTCCTG
1051  AACCCGTGTG CCAACATGAA GGTGTGCAAT GAGGACCAGA CCAACTGCAC
1101  AGTGCCCACT TACCCCTCGT GCCGTGACAG CGAGACCTTC AGCACCTTCC
1151  TCCTGGACCT GTTTAAGCTG ACCATCGGCA TGGGCGACCT GGAGATGCTG
1201  AGCAGCACCA AGTACCCCGT GGTCTTCATC ATCCTGCTGG TGACCTACAT
1251  CATCCTCACC TTTGTGCTGC TCCTCAACAT GCTCATTGCC CTCATGGGCG
1301  AGACAGTGGG CCAGGTCTCC AAGGAGAGCA AGCACATCTG GAAGCTGCAG
1351  TGGGCCACCA CCATCCTGGA CATTGAGCGC TCCTTCCCCG TATTCCTGAG
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1401   GAAGGCCTTC CGCTCTGGGG AGATGGTCAC CGTGGGCAAG AGCTCGGACG

1451   GCACTCCTGA CCGCAGGTGG TGCTTCAGGG TGGATGAGGT GAACTGGTCT

1501   yACTGGAACC AGAACTTGGG CATCATCAAC GAGGACCCGG GCAAGAATGA

1551   GACCTACCAG TATTATGGCT TCTCGCATAC CGTGGGCCGC CTCCGCAGGG

1601   ATCGCTGGTC CTCGGTGGTA CCCCGCGTGG TGGAACTGAA CAAGAACTCG

1651   AACCCGGACG AGGTGGTGGT GCCTCTGGAC AGCATGGGGA ACCCCGCTG

1701   CGATGGCCAC CAGCAGGGTT ACCCCCGCAA GTGGAGGACT GATGACGCCC

1751   CGCTCTAGGG ACTGCAGCCC AGCCCCAGCT TCTCTGCCCA CTCATTTCTA

1801   GTCCAGCCGC ATTTCAGCAG TGCCTTCTGG GGTGTCCCCC CACACCCTGC

1851   TTTGGCCCCA GAGGCGAGGG ACCAGTGGAG GTGCCAGGGA GGCCCCAGGA

1901   CCCTGTGGTC CCCTGGCTCT GCCTCCCCAC CCTGGGGTGG GGGCTCCCGG

1951   CCACCTGTCT TGCTCCTATG GAGTCACATA AGCCAACGCC AGAGCCCCTC

2001   CACCTCAGGC CCCAGCCCCT GCCTCTCCAT TATTTATTTG CTCTGCTCTC

2051   AGGAAGCGAC GTGACCCCTG CCCCAGCTGG AACCTGGCAG AGGCCTTAGG

2101   ACCCCGTTCC AAGTGCACTG CCCGGCCAAG CCCCAGCCTC AGCCTGCGCC

2151   TGAGCTGCAT GCGCCACCAT TTTTGGCAGC GTGGCAGCTT TGCAAGGGGC

2201   TGGGGCCCTC GGCGTGGGGC CATGCCTTCT GTGTGTTCTG TAGTGTCTGG

2251   GATTTGCCGG TGCTCAATAA ATGTTTATTC ATTGAAAAAA AAAAAAAAA
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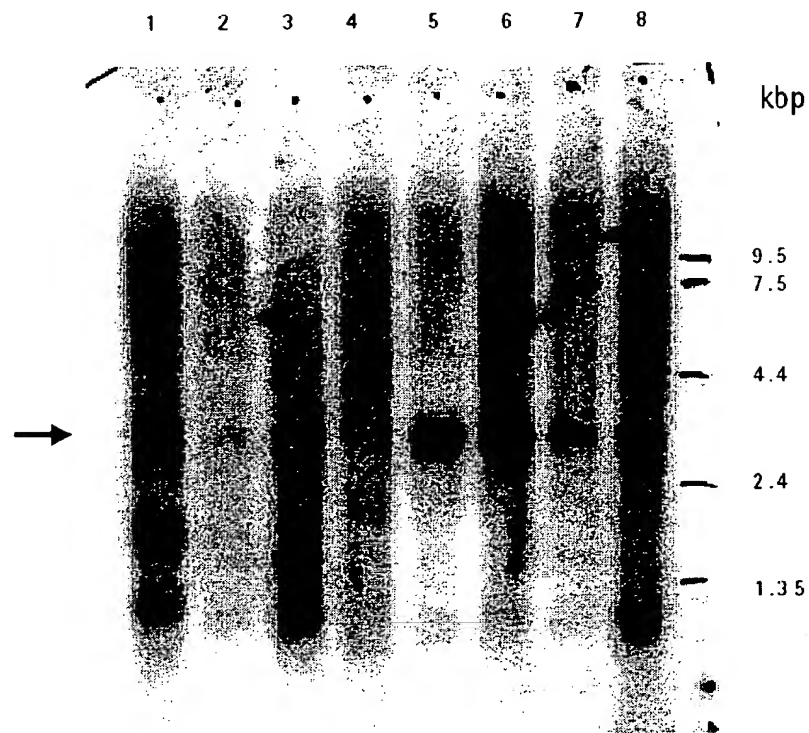Figure 10: Hybridisation of northern blot with hVR2 probe

Lane 1: Spleen   Lane 5: Ovary
Lane 2: Thymus   Lane 6: Small intestine
Lane 3: Prostate   Lane 7: Colon
Lane 4: Testis   Lane 8: Peripheral blood leukocyt

Figure 11: Hybridisation of northern blot with hVR2 probe

Lane 1: Pancreas   Lane 5: Lung
Lane 2: Kidney   Lane 6: Placenta
Lane 3: Skeletal muscle Lane 7: Brain
Lane 4: Liver   Lane 8: Heart

Figure 12: Hybridisation of a northern blot with hVR3
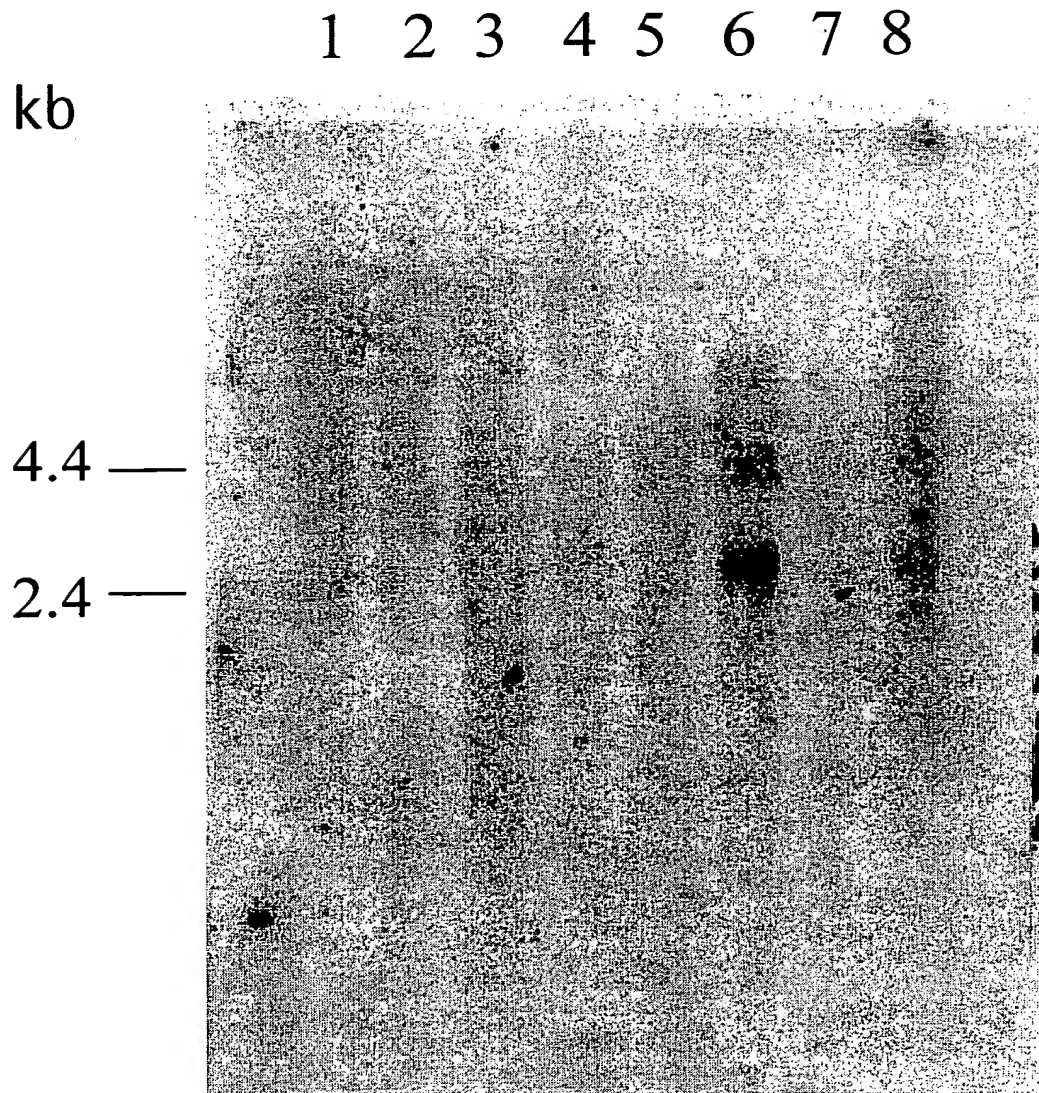
kb

1 2 3 4 5 6 7

4.4 —

2.4 —
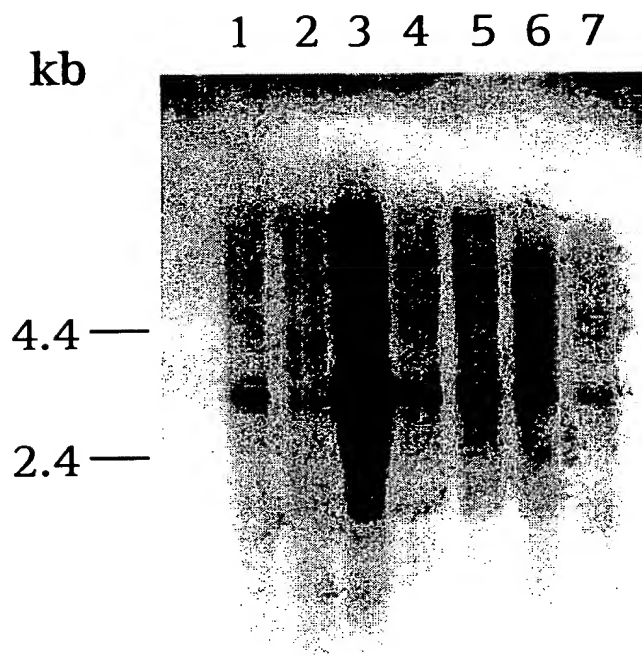
Lane 1: Bone marrow
Lane 2: Adrenal Gland
Lane 3: Trachea
Lane 4: Lymph node

Lane 5: Spinal cord
Lane 6: Thyroid
Lane 7: Stomach

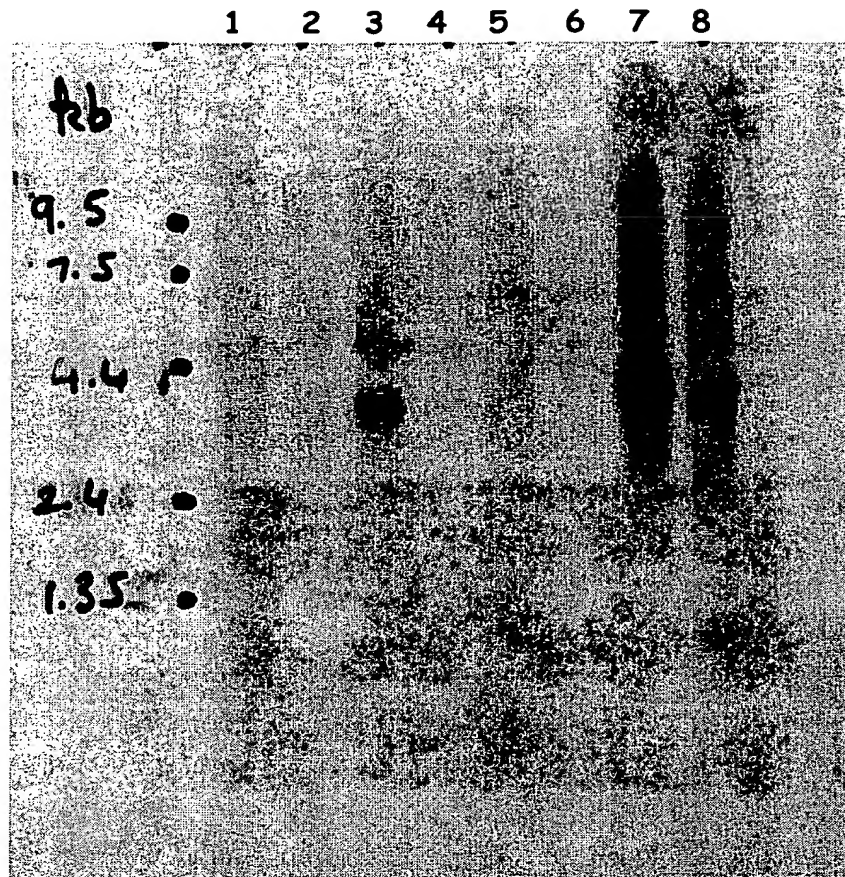**Figure 13: Hybridisation of northern blot with hVR3 probe**

Lane 1: Peripheral Blood Leukocyte
Lane 2: Colon
Lane 3: Small Intestine
Lane 4: Uterus
Lane 5: Testis
Lane 6: Prostate
Lane 7: Thyroid
Lane 8: Spleen

Figure 14: Hybridisation of a multi-tissue northern blot with the hVR3 prob

Lane 1: Heart   Lane 5: Liver
Lane 2: Brain   Lane 6: Skeletal Muscle
Lane 3: Placenta   Lane 7: Kidney
Lane 4: Lung   Lane 8: Pancreas

Figure 15

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